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HEMATOLOGIC AND SELECTED HEPATIC CHANGES PRODUCED BY SUBSTITUTE--ETC(U)
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(6) HEMATOLOGIC AND SELECTED HEPATIC CHANGES PRODUCED BY SUBSTITUTED p-BENSOQUINONES IN THE RAT,

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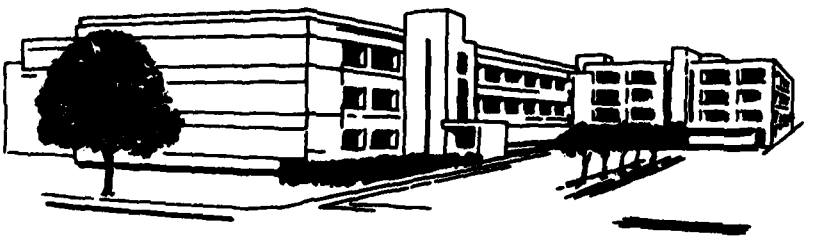

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Comittee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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HEMATOLOGIC AND SELECTED HEPATIC CHANGES PRODUCED BY SUBSTITUTED p-BENZOQUINONES IN THE RAT

Benzoquinones are secreted by a number of tenebrionid flour beetles of which the Tribolium spp. are the most prominent food pests (1). The major components of the odoriferous secretion have been identified as 2-methyl-1,4-benzoquinone (MBQ) and 2-ethyl-1,4-benzoquinone (EBQ) (1-5). Benzoquinones are highly reactive compounds and evidence indicates that they are acutely toxic and perhaps carcinogenic to laboratory animals (6,7). Poisoning by hydroquinone-quinone-systems in man is characterized by jaundice, anemia, hemoglobinuria, and cachexia (8). In previous studies, we found that benzoquinone toxicity in rats resulted in respiratory depression, skin blanching, and cyanosis before death (9,10).

Since respiratory impairment may be due in part to inadequate blood oxygen, we conducted preliminary studies to examine the effects of substituted benzoquinones on the heme in red blood cells and in hepatic microsomal enzymes.

METHODS

All chemicals were of the highest purity available. Agar (No. 0140) was obtained from Difco Laboratories, Detroit, MI. MBQ was obtained from J.T. Baker Chemical Co., Phillipsburg, NJ. EBQ was synthesized following the procedures of Ladisch and Suter (6). The adult male Sprague-Dawley rats (50-days old, weighing 200 ± 50 g) were obtained from Charles River, Wilmington, MA. Upon arrival, each animal was examined for abnormalities and normal rats were randomly housed in stainless steel wire-bottomed cages, one to a cage, and fed a stock diet (Rodent Laboratory Chow #5001, Ralston Purina Co., St. Louis, MO) ad libitum. The rats were divided into three groups of five animals each. The animals in each group were dosed daily with either the aqueous vehicle (0.25% agar), MBQ, or EBQ for four days before killing. The benzoquinones, suspended by trituration in 0.25% agar at concentrations of 90 mg MBQ/5 ml and 110 mg EBQ/5 ml, were administered at the

LD_{05} level (9,10). Dosage was by oral intubation at a constant volume of 5 ml/kg body weight.

Twenty-four hours after the last dosing the rats were weighed and killed by decapitation. Blood from decapitation was collected into tubes containing EDTA and kept at 4 C for subsequent hematologic measurements. The livers were excised, weighed, perfused with ice-cold 1.15% KCl, and minced. Liver preparation, subcellular fractionation, and hepatic cytochrome-drug assays were done as previously described (11,12). Nonprotein sulfhydryls were measured in whole liver homogenates (13) and glutathione. GSH-s-transferase activity was measured in the post-100,000 xG supernatant (cytosol) (14).

Data were analyzed statistically by the Student's t test or by analysis of variance.

RESULTS

After four days of dosing with substituted p-benzoquinone the rats appeared passive and listless compared to agar-treated controls. Table 1 shows that blood leukocytes were significantly increased (67%) after MBQ or EBQ treatment and that there was also a tendency for the differential lymphocyte to neutrophil ratio to increase compared to controls. In general, Table 1 shows that MBQ and EBQ treatment resulted in fewer erythrocytes (8.4% and 10.7%, respectively) and lower hemoglobin and hematocrits than agar-treated controls (5.6 to 14.8%).

Table 1. Hematology on rats treated with substituted p-benzoquinones*

Compound	WBC $x10^3$	RBC 10^6	HGB gm	HCT %	Differential WBC Counts, % Lymphocytes	Neutrophils
Control	10.3 ± 0.8	6.45 ± 0.18	14.3 ± 0.4	39.1 ± 0.8	24 ± 3	70 ± 4
MBQ	17.2 ± 2.1†	5.91 ± 0.26	13.5 ± 0.7	34.7 ± 1.8	36 ± 4†	61 ± 4
EBQ	17.2 ± 1.2†	5.76 ± 0.20†	13.0 ± 0.3†	33.3 ± 1.0†	34 ± 4	63 ± 4

* Mean S.E.M.; N = 5; MBQ = 2-methyl-1,4-benzoquinone; EBQ = 2-ethyl-1,4-benzoquinone; WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit.

† $P < 0.05$ compared to control groups.

Table 2 demonstrates that the treatment of rats with substituted p-benzoquinones resulted in no statistical change of body weight, liver weight, or liver microsomal protein. Table 3 shows that MBQ and EBQ

Table 2. Body and liver weight and protein content of liver microsomal protein in rats treated with substituted p-benzoquinones*

Compound	Body Weight g	Liver Weight g	Microsomal Protein mg/g
Control	285 ± 10.0	9.5 ± 0.59	13.78 ± 1.09
MBQ	288 ± 05.0	10.5 ± 0.69	14.87 ± 0.87
EBQ	274 ± 06.6	10.3 ± 0.43	16.68 ± 1.07

* Mean ± S.E.M.; N = 5; MBQ = 2-methyl-1,4-benzoquinone; EBQ = 2-ethyl-1,4-benzoquinone.

Table 3. Liver microsomal cytochrome levels in rats treated with substituted p-benzoquinones*

Compound	Cytochromes (nmoles/mg protein) P-450	b_5
Control	1.221 ± 0.079	0.351 ± 0.019
MBQ	1.076 ± 0.065	0.270 ± 0.010†
EBQ	0.926 ± 0.106†	0.258 ± 0.024†

* Mean ± S.E.M.; N = 5; MBQ = 2-methyl-1,4-benzoquinone; EBQ = 2-ethyl-1,4-benzoquinone.

† P ± 0.05 compared to control groups.

treatment of rats resulted in decreases of both cytochrome P-450 and cytochrome b_5 hepatic microsomal heme proteins (23.1 to 26.5%). Table 4 documents that the cytochrome P-450 dependent mixed-function oxidase activity of aniline hydroxylase decreased significantly after treatment with substituted p-benzoquinones. Similar trends were also found for aminopyrine demethylase activity after treatment with MBQ and EBQ.

Table 4. Liver microsomal mixed-function oxidase activity in rats treated with substituted p-benzoquinones*

Compound	Aminopurine N-demethylase (nmole/mg protein)	Aniline hydroxylase (nmole/mg protein)
Control	5.164 ± 0.458	0.701 ± 0.041
MBQ	4.718 ± 0.333	0.521 ± 0.031†
EBQ	4.970 ± 0.373	0.402 ± 0.050†

* Mean ± S.E.M.; N = 5; MBQ = 2-methyl-1,4-benzoquinone; EBQ = 2-ethyl-1,4-benzoquinone.

† $P < 0.05$ compared to control group

In contrast to the suggested mechanism of action (15), Table 5 shows that p-benzoquinones do not reduce liver sulfhydryls in vivo. In addition, MBQ and EBQ had no effect on cytosolic GSH-s-transferase activity.

Table 5. Liver nonprotein sulfhydryls and glutathione-s-transferase activity in rats treated with substituted p-benzoquinones*

Compound	Nonprotein sulfhydryls (nmole/mg protein)	GSH-s-transferase (μmole/mg protein)
Control	3.53 ± 0.152	0.845 ± 0.051
MBQ	4.25 ± 0.514	0.764 ± 0.035
EBQ	3.92 ± 0.186	0.890 ± 0.076

* Mean ± S.E.M.; N = 5; MBQ = 2-methyl-1,4-benzoquinone; EBQ = 2-ethyl-1,4 benzoquinone.

DISCUSSION

Several *in vitro* reactions have been described as explanation for the p-benzoquinone toxicity to animals. Synthetic EBQ and quinone mixtures isolated from *Tribolium castaneum* are known to form quinone conjugates with -NH and -SH groups of amino acids (15), proteins (15, 16) and similar compounds. The conjugates with amino acids are intensely colored and often fluorescent (15,17). Irreversible formation of -SH groups seems unlikely since we could find no evidence of loss of total nonprotein sulfhydryls (Table 5) in liver tissues of rats treated with MBQ or EBQ. In addition, there was no influence of substituted p-benzoquinones on GSH-s-transferase activity, a cytosolic enzyme dependent on GSH.

Microsomal heme protein and cytochrome P-450, as well as the corresponding mixed-function oxidase activity and blood heme parameters, seem to be markedly influenced by substituted p-benzoquinone treatment. This is consistent with the hydroquinone-quinone poisoning, characterized in man by anemia and hemoglobinuria (8). Our observations of decreased heme-containing proteins and blood heme after MBQ and EBQ treatments are also consistent with our previous observations of cyanosis, extreme skin blanching, and depressed respiration in rats treated with p-benzoquinone and substituted p-benzoquinones (9,10).

Affinity of benzoquinones for amino acids and proteins may result in decreased synthesis or increased degradation of heme or apoprotein. Ladisch and Suter (16) have also suggested that the affinity of the benzoquinones for protein is responsible for their carcinogenic properties; however, further investigation is needed to determine any resultant alterations of biochemical function which may be responsible for the observed toxicity of these quinones.

SUMMARY

Rats were given daily oral doses of 2-methyl-1,4-benzoquinone (MBQ) or 2-ethyl-1,4-benzoquinone (EBQ) at the LD_{05} level for four days. There were 5.6 to 14.8% decreases of blood heme paramaters in rats treated with substituted p-benzoquinone compared to agar-treated controls. Benzoquinone treatment resulted in 67% increases of blood leukocyte counts. Hepatic microsomal cytochrome P-450 and cytochrome b_5 contents were decreased 11.9 to 26.5% in benzoquinone-treated rats. Aniline hydroxylase activity decreased 25.7 to 42.7%. There was no change in liver weight, microsomal protein, nonprotein sulfhydryls, or GSH-s-transferase activity. The results of this study suggest that substituted p-benzoquinones may have exerted their toxic action in rats by altering heme metabolism.

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